

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

In re Application of:

Karla ROBOTTI

Confirmation No. 2898

Serial No. 10/072,525

Group Art Unit 1636

Filing Date: February 5, 2002

Examiner Quang NGUYEN

Title: METHOD OF IMMOBILIZING BIOLOGICALLY ACTIVE MOLECULES FOR ASSAY
PURPOSES IN A MICROFLUIDIC FORMAT

APPEAL BRIEF

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APPEAL BRIEF

Pursuant to 35 U.S.C. § 134(a) and 37 C.F.R. § 1.192, the final rejection of pending claims 9, 14, 15, 28-32, 37-40, 45-56, 58, and 59 of this application is appealed. These claims were finally rejected under 35 U.S.C. § 112, second paragraph, and 35 U.S.C. § 103(a) in the final Office Action issued August 11, 2004. A Notice of Appeal was timely filed on December 20, 2004. This appeal brief is accompanied by an authorization to debit appellant's Deposit Account No. 50-1078 (Agilent Technologies, Inc.) for the required one-month extension of time fee.

(1) Real Party in Interest:

The real party in interest is Agilent Technologies, Inc., by way of an assignment from the inventors to Agilent Technologies, Inc., recorded with the U.S. Patent and Trademark Office.

(2) Related Appeals and Interferences:

There are no related appeals and interferences for this matter.

(3) Status of Claims:

Claims 1-6, 9, 14-21, 24, and 26-59 are pending and claims 7, 8, 10-13, 22, 23, and 25 have been previously canceled. Claim 44 has been allowed. Claims 9, 14, 15, 28-32, 37-40, 45-56, 58, and 59 have been finally rejected as follows:

1. Claims 9, 14, 15, 28-32, and 37-40 stand rejected under 35 U.S.C. 112, second paragraph as being indefinite;
2. Claims 9, 14, 15, 28-32, and 37-40 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Dunn et al. (U.S. Patent No. 5,200,334) in view of Reetz et al. (*Biotechnology and Bioengineering* 9:527-534 (1994)); and
3. Claims 45-56, 58, and 59 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Dunn et al. in view of Avnir et al. (U.S. Patent No. 5,300,564), Swedberg et al. (U.S. Patent No. 6,240,790), and Freeman et al. (U.S. Patent No. 6,194,900).

In addition, certain claims have been objected to as follows:

1. Claims 1-6, 9, 14-21, 24, and 26-43 stand objected to due to the inclusion of the phrase "in step (b) " without antecedent basis in each of independent claims 1 and 9; and
2. Claims 1-6, 16-21, 24, 26, 27, 33-36, and 41-43 stand objected to for unstated reasons (Office Action Summary page, item number 7, Office Action dated August 11, 2004).

Because these objections do not involve rejections of the claims, they are not addressed herein. However, in the interest of clarifying the record, appellant notes that the first objection may be readily obviated by deletion of the phrase "in step (b)" in each of claims 1 and 9. With regard to the second objection, appellant does not consider that any additional grounds for objection have been cited by the Examiner that would necessitate further comment and/or amendment by appellant.

(4) Status of Amendments:

Two after-final amendments were submitted in response to the final rejection dated August 11, 2004. The first after-final amendment was filed on October 12, 2004 and the second was filed on December 13, 2004. Both after-final amendments were denied entry by the Examiner in the Advisory Actions issued November 15, 2004 and February 8, 2005, respectively.

(5) Summary of the Invention:

The present relates generally to a method for immobilizing biomolecules without affecting their biological function. More specifically, the invention relates to immobilization of biomolecules in porous, inorganic matrices, and to methods of using the immobilized biomolecules in various contexts. The invention additionally relates to microfluidic systems wherein the immobilized biomolecules are

contained within microchannels, microcolumns, or the like, for example, for performing high throughput screening in a microfluidic format.

Molecules and cellular structures have previously been immobilized by entrapment; however, the entrapment was usually accomplished using organic polymers such as polyvinyl alcohol or polyacrylic acid as the trapping medium. Such organic polymer based entrapment often resulted in weaker gel networks that broke apart and allowed the biomolecule to leach from the gel.

As is described in detail in appellant's specification, the devices and methods of the invention provide a solution to this and other problems with prior art applications by, in part, providing a method of immobilizing biomolecules through entrapment within an inorganic silicate or sol-gel for purposes of high throughput screening. The entrapped biomolecules are not altered by covalent modification, and are not merely adsorbed, and therefore will not leach from the matrix. The biomolecules entrapped within the sol-gel may be used to perform binding or enzyme activity assays, for example, on a "chip" rather than keeping them as an off-line process that feeds into a micro device. The immobile molecule may be placed into a chamber on-device or may be formed *in situ* as a part of the device.

The present invention further provides devices and methods for performing high throughput screening using the biomolecule containing matrices.

Although not limited thereto, as set forth in independent claim 9 on appeal, the invention concerns a method for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device, said method comprising:

forming an aqueous composition comprising a tetraalkyl orthosilicate and a silane, wherein the silane is substituted with a C₈-C₂₄ alkyl group and substituted with at least two leaving groups selected from OR and halo, mixed with an acidified oxide salt solution;

adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;

shaping the gel produced in step (b) into a final form; and

aging the gel;

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic matrix is formed *in situ*.

As set forth in independent claim 45 on appeal, the invention also concerns a method of preparing a microanalytical device comprising forming a sol-gel comprising an entrapped biological molecule, wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, or fiber and wherein the sol-gel is placed in or on the microanalytical device.

Though again not limited thereto, the invention further concerns a method of using a microanalytical device comprising a sol-gel comprising an entrapped biological molecule, comprising forming the sol-gel into a bed within the microanalytical device or on the surface of the microanalytical device, applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed, as recited in independent claim 46 on appeal.

As further set forth in independent claim 58 on appeal, the invention also concerns improvements in a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.

(6) The Cited References:

1. Dunn et al. (U.S. Patent No. 5,200,334)

U.S. Patent No. 5,200,334 to Dunn et al. relates to the entrapment of enzymes in sol-gels, wherein a metal alkoxide is mixed with water and exposed to ultrasonic energy at a defined pH to form a single phase solution which is then buffered to a pH between about 5 and 7. The buffered solution is then mixed with the active biological material and the resultant gel is aged and dried. Dunn et al. reports that the dried product is a transparent porous glass with substantially all of the added active biological material entrapped therein, and that the biological material retained a high level of activity.

2. Reetz et al. (Biotechnology and Bioengineering 9:527-534 (1994))

Reetz et al. relates to the immobilization of lipases by entrapment in hydrophobic sol-gel materials, such as hydrophobic silica gels prepared by hydrolysis of alkyl-substituted silanes in the presence of the enzyme. The method is said to be applicable to lipases, yielding immobilized lipases with enhanced esterification activities.

Although Reetz et al. mentions that the immobilized lipase may be crushed and ground, there is no indication that a particular particle size or range of particle sizes is produced. In addition, Reetz et al. generally refers to the morphology (page 532) as well as the porosity and pore diameter (page 530) of the sol-gel but does not indicate that the lipases are entrapped within pores of the gel.

3. Avnir et al. (U.S. Patent No. 5,300,564)

U.S. Patent No. 5,300,564 to Avnir et al. describes a proposed method of obtaining a chemical interaction between at least one reagent trapped in sol-gel glass by doping it with the reagent, and diffusible solutes or components in an adjacent liquid or gas phase, wherein the reagents, the solutes or

the components can be any organic or inorganic compounds or materials of biological origin including enzymes. The doped sol-gel glass in various forms may be useful as an analytical test, chromatographic medium, sensor, catalyst or biocatalyst, electrode or enzyme electrode, or other detection device.

4. *Swedberg et al. (U.S. Patent No. 6,240,790)*

U.S. Patent No. 6,240,790 to Swedberg et al. relates to a microanalysis device having a plurality of sample processing compartments for use in liquid phase analysis. The device is formed by microfabrication of microstructures in support substrates.

5. *Freeman et al. (U.S. Patent No. 6,194,900)*

U.S. Patent No. 6,194,900 to Freeman et al. relates to a miniaturized total analysis system with an in-line NMR detection compartment and an NMR rf microcoil detector for use in liquid phase analysis. The device is formed by microfabrication of microstructures in support substrates.

(7) Issues on Appeal:

The following issues are to be considered in this appeal:

1. Whether claims 9, 14, 15, 28-32, and 37-40 are unpatentable as not satisfying the definiteness requirement of 35 U.S.C. § 112, second paragraph;
2. Whether claims 9, 14, 15, 28-32, and 37-40 are obvious under 35 U.S.C. §103(a) over Dunn et al. (U.S. Patent No. 5,200,334) in view of Reetz et al. (*Biotechnology and Bioengineering* 9:527-534 (1994)); and
3. Whether claims 45-56, 58, and 59 are obvious under 35 U.S.C. §103(a) over Dunn et al. in view of Avnir et al. (U.S. Patent No. 5,300,564), Swedberg et al. (U.S. Patent No. 6,240,790), and Freeman et al. (U.S. Patent No. 6,194,900).

(8) Grouping of Claims:

For purposes of this appeal, the claims on appeal do not stand or fall together. Appellant submits that claims 9, 14, 15, 28-32, and 37-40 are separately patentable from claims 45-56, 58, and 59 at least for the reason that the former group of claims includes the requirement that the porous inorganic matrix is formed in situ. This feature of appellant's invention is not recited in independent claims 45, 46, and 58 and is not disclosed or suggested by the references cited as a basis for rejection of the claims. Accordingly, appellant considers that this feature provides separate patentability to claims 9, 14, 15, 28-32, and 37-40.

(9) Argument:

I. *The Definiteness Rejection under 35 U.S.C. § 112, Second Paragraph*

A. *The Legal Standard for the Definiteness Requirement*

The second paragraph of 35 U.S.C. § 112 is directed to requirements for the claims, and reads as follows:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

There are two separate requirements set forth in this paragraph:

- (a) the claims must set forth the subject matter that applicants regard as their invention; and
- (b) the claims must particularly point out and distinctly define the metes and bounds of the subject matter that will be protected by the patent grant.

The test for definiteness under 35 U.S.C. 112, second paragraph, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986). In reviewing a claim for compliance with 35 U.S.C. 112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. 112, second paragraph, by providing clear warning to others as to what constitutes infringement of the patent. See, e.g., *Solomon v. Kimberly-Clark Corp.*, 216 F.3d 1372, 1379, 55 USPQ2d 1279, 1283 (Fed. Cir. 2000); and *In re Larsen*, No. 01-1092 (Fed. Cir. May 9, 2001) (unpublished) (The preamble of the Larsen claim recited only a hanger and a loop but the body of the claim positively recited a linear member. The court observed that the totality of all the limitations of the claim and their interaction with each other must be considered to ascertain the inventor's contribution to the art. Upon review of the claim in its entirety, the court concluded that the claim at issue apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. 112 paragraph 2.).

Further, as noted in the Manual of Patent Examining Procedure (MPEP) at section 2173.05(e),

... the failure to provide explicit antecedent basis for terms does not always render a claim indefinite. If the scope of a claim would be reasonably ascertainable by those skilled in the art, then the claim is not indefinite. *Ex parte Porter*, 25 USPQ2d 1144, 1145 (Bd. Pat. App. & Inter. 1992). Inherent components of elements recited have antecedent basis in the recitation of the components themselves (citing, *Bose Corp. v. JBL, Inc.*, 274 F.3d 1354, 1359, 61 USPQ2d 1216, 1218-19 (Fed. Cir 2001)).

B. The Examiner's Statement of the Rejection

In the Office Action dated August 11, 2004, claims 9, 14, 15, 28-32, and 37-40 were rejected under 35 U.S.C. §112, second paragraph as being indefinite, for the following reasons:

In claim 9 and its dependent claims, there is no linkage between the method steps being recited and the preamble "immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device". Therefore, the metes and bounds of the claims are not clearly determined.

(Office Action dated August 11, 2004, page 3, emphasis in original)

C. Appellant's Arguments Against the Rejection

(1) Claim 9 and Claims Dependent Therefrom Satisfy the Definiteness Requirement of 35 U.S.C. §112, Second Paragraph

For convenience, the text of claim 9 is reproduced below:

A method for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device, said method comprising:

forming an aqueous composition comprising a tetraalkyl orthosilicate and a silane, wherein the silane is substituted with a C₈-C₂₄ alkyl group and substituted with at least two leaving groups selected from OR and halo, mixed with an acidified oxide salt solution;

adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;

shaping the gel produced in step (b) into a final form; and

aging the gel;

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic matrix is formed in situ.

As set forth in the preamble of claim 9, appellant's claimed method is "for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device." By comparison, the body of the claim reciting the method steps refers to the in situ formation of the porous inorganic matrix ("wherein the porous inorganic matrix is formed in situ") and that the "biological molecule is entrapped within pores of the gel." Upon close comparison of the language of the preamble and the body of the claim, the skilled artisan would reasonably understand that the biological material referred to is entrapped (i.e., immobilized) in a porous inorganic matrix.

As may be readily noted, appellant's claim 9 does not explicitly recite that the matrix is incorporated "into a microanalytical device" in the body of the claim. However, the skilled artisan would reasonably understand that claimed method is directed to the incorporation of the matrix into a microanalytical device at least for the reason that this language would be inferred from the preamble. In

addition, since the claim must be read as a whole, not just as individual separable parts (e.g., preamble and body of the claim), and must also be read in light of the specification, the skilled artisan would reasonably understand that the claimed method is directed to immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device. As such, appellant's claimed method would be understood to include incorporation of the matrix into such a device.

Appellant also notes that the recitation "the porous inorganic matrix is formed *in situ*" was added in an Amendment filed May 20, 2004. In that document, it was noted that support for this amendment was present at least at page 6, lines 6-7 and page 11, lines 1-4 of the specification. More specifically, the meaning of this language, as supported by the specification, is clearly that the matrix is "formed as an integral part of a microfluidic device" (page 6, lines 6-7 of the specification). Appellant further notes that additional support for the *in situ* formation of the matrix and the immobilized biomolecule is present in the specification at page 7, lines 15-19 ("The immobile molecule may be placed into a chamber on-device or may be formed *in situ* as a part of the device."). As such, the skilled artisan, being familiar with the entire disclosure of application and the meaning of the claims, would reasonably understand that the inorganic matrix is incorporated into a microanalytical device, as is recited in the preamble of claim 9. (Please also note the use of the terms "microfluidic device" and "microanalytical devices" in the specification at page 23, lines 7-12, in that the term "microfluidic devices" refers to two or three dimensional microanalytical devices).

In addition, appellant respectfully submits that adequate notice of the meaning and scope of the claims has been provided in accordance with the notice function required by 35 U.S.C. 112, second paragraph.

For at least the foregoing reasons, appellant respectfully submits that the rejection of claims 9, 14, 15, 28-32, and 37-40 under 35 U.S.C. §112, second paragraph is in error and requests that the rejection be reversed by the Board of Appeals.

II. The Obviousness Rejections under 35 U.S.C. § 103(a)

A. The Legal Standard for Obviousness

35 U.S.C. 103(a) reads as follows:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary

skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

A finding of *prima facie* obviousness is a procedural tool which, as used in patent examination, means not only that the evidence of the prior art would reasonably allow the conclusion the Examiner seeks, but also that the prior art compels such a conclusion if the applicant produces no evidence or argument to rebut it. *In re Spada*, 911 F.2d 705, 15 USPQ2d 1655 (Fed. Cir. 1990). To establish a *prima facie* case of obviousness, three criteria must be met: first, the prior art reference must teach or suggest the desirability of the claimed combination; second, the Office must show that the ordinary artisan would be motivated to modify the reference or to combine the reference teachings; and third, there must be a showing that the ordinary artisan would have a reasonable expectation of success at arriving at the claimed combination based *solely* on the teachings of the cited prior art reference. *In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453 (Fed. Cir. 1998); *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991); *In re Deminski*, 796 F.2d 436, 230 USPQ 313 (Fed. Cir. 1986).

B. The Examiner's Statement of the Rejection

- (1) *Rejection of claims 9, 14, 15, 28-32, and 37-40 as obvious under 35 U.S.C. §103(a) over Dunn et al. (U.S. Patent No. 5,200,334, hereinafter "Dunn et al.") in view of Reetz et al. (Biotechnology and Bioengineering 9:527-534 (1994), hereinafter "Reetz et al.").*

In the Office Action dated February 24, 2004, claims 1, 9, 12-14, 15, 28-32, and 37-40 were rejected under 35 U.S.C. §103(a) as being obvious over Dunn et al. in view of Reetz et al., for the following reasons:

Dunn et al. teach a process for the production of a porous, transparent sol-gel glass containing an alcohol sensitive active biological material entrapped therein comprising: (a) forming a single phase sol by mixing a metal alkoxide in a non-alcoholic medium comprising water and an acid catalyst in a container exposed to ultrasonic energy, the mixture having a pH not greater than about 2; (b) removing the ultrasonic energy and raising the pH of the sol to about 5 to 7 by the addition of a buffering agent; (c) adding an alcohol sensitive biological material to the buffered sol; (d) forming a gel and allowing the gel to age; and (e) allowing at least a portion of the water in the gel to evaporate so that the volume of the product produced in step (d) is decreased and the active biological material is trapped in a monolith of the gel having a reduced volume (see abstract, Fig.1 and claim 1). Although exemplified method utilizes tetramethylorthosilicate (TMOS), and proteins (e.g., RNase A, proteases, hemoglobin, cytochrome c, metal ion binders, see sol. 3, lines 38-59 and Table 1) as active biological materials, however other silicon alkoxides such as tetraethylorthosilicate (TEOS) and other active silicon compounds as well as other metal alkoxides (not limited to aluminum, titanium, zirconium, vanadium, sodium, calcium and boron or combinations thereof) can be used (col. 2, line 60 continues to line 10 of col. 3). Dunn et al. further teach that it would be highly advantageous to encapsulate enzymes in a porous, transparent

glass structure, such as structures prepared by the sol-gel process. Such an encapsulation would be significantly easier to miniaturize and would be far less cumbersome and far more reliable than membrane encapsulating systems. Furthermore, enzyme encapsulation within a transparent glass structure would allow for the monitoring of many enzymatic reactions by using simple, photometric monitoring systems (col.1, lines 27-36)

(Office Action dated February 24, 2004, pages 8-9, emphasis in original)

As further stated in the Office Action dated February 24, 2004:

The teachings of Dunn et al. have already been presented above. However, Dunn et al. do not specifically teach a method wherein the sol comprises a tetralkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo, or wherein the silane is substituted with a C₈-C₂₄ alkyl group or wherein the alkyl group is C₁₈.

However at the filing date of the present application Reetz et al. teach that lipase activity in gels from a mixture of tetramethoxysilane (TMOS) and alkyltrimethoxysilanes R₃Si(OCH₃)₃ was dramatically enhanced with increasing amount and alkyl chain length of the hydrophobic silanes, including the alkyl group C₁₈ (page 259, right-handed column, first complete paragraph and Figure 1).

Accordingly, at the effective filing date of the present application, it would have obvious for an ordinary skilled artisan in the art to modify the method taught by Dunn et al. by further introducing a substituted silane as recited in the sol in light of the teachings of Reetz et al. due to the stabilizing effect on entrapped lipase by increasing amount and alkyl chain length of the hydrophobic silanes.

An ordinary skilled artisan would have been motivated to carry out the above modification because increasing amount and alkyl chain length of the hydrophobic silanes, including the alkyl group C₁₈ enhance lipase-doped sol-gel as taught by Reetz et al.

An ordinary skilled artisan would have a reasonable expectation of success based on the teachings of Dunn et al. and Reetz et al., as well a [sic] high level of skill of an ordinary skilled artisan in the art.

Accordingly, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

(Office Action dated February 24, 2004, pages 12-13)

In the Office Action dated August 11, 2004, claims 9, 14, 15, 28-32, and 37-40 were rejected under 35 U.S.C. §103(a) as being obvious over Dunn et al. in view of Reetz et al. for the reasons set forth in the Office Action dated February 24, 2004, and for the following additional reasons:

Applicant's argument related to the above rejection in the Amendment filed on 5/20/04 (pages 15-16) have been fully considered, but it is respectfully found to be unpersuasive.

Applicant argues mainly that neither Dunn nor Reetz teaches or suggests forming a sol in situ in a microanalytical device.

Please note that the rejection is maintained because the combined teachings of Dunn and Reetz have the same method steps as those recited in the amended claim 9. Furthermore, the term "the porous inorganic material is formed *in situ*" includes the the [sic] formation of a porous inorganic material in any reaction vessel.

Accordingly, amended claims 9, 14-15, 28-32 and 37-40 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Dunn et al. in view of Reetz et al. for the reasons of record.

(Office Action dated August 11, 2004, page 3, emphasis in original)

- (2) *Rejection of claims 45-56, 58, and 59 as obvious under 35 U.S.C. §103(a) over Dunn et al. in view of Avnir et al. (U.S. Patent No. 5,300,564, hereinafter "Avnir et al."), Swedberg et al. (U.S. Patent No. 6,240,790, hereinafter "Swedberg et al."), and Freeman et al. (U.S. Patent No. 6,194,900, hereinafter "Freeman et al.").*

In the Office Action dated February 24, 2004, claims 45-56, 58, and 59 were rejected under 35 U.S.C. §103(a) as being obvious over Dunn et al. in view of Avnir et al., Swedberg et al., and Freeman et al., for the following reasons:

Dunn et al. teach a process for the production of a porous, transparent sol-gel glass containing an alcohol sensitive active biological material entrapped therein, including in the form of thin films as small as 1000 Angstroms thick or shaped gels having dimensions in its smallest direction of at least 0.5 cm or a monolith (see Summary and col. 2, lines 1-5). Exemplified method utilizes tetramethylorthosilicate (TMOS), and proteins (e.g., RNase A, proteases, hemoglobin, cytochrome c, metal ion binders, see col. 3, lines 38-59 and Table 1) as active biological materials. Dunn et al. further teach that encapsulated or entrapped enzymes are used with increasing frequency as micro-catalysts and analytical devices of very high sensitivity, and that enzymes have been enclosed in membranes systems and used as high-sensitivity monitoring devices. However, such membrane systems are cumbersome and difficult to miniaturize. Therefore, it would be highly advantageous to encapsulate enzymes in a porous, transparent glass structure, such as structures prepared by the sol-gel process. Such an encapsulation would be significantly easier to miniaturize and would be far less cumbersome and far more reliable than membrane encapsulating systems (col. 1, lines 27036[sic]). Additionally, enzyme encapsulation within a transparent glass structure would allow for the monitoring of many enzymatic reactions by using simple, photometric monitoring systems (col. 1, lines 27-36). Because of the light transmission characteristics of the glasses, UV, IR and visible light optical spectroscopy as well as fluorescence, luminescence, absorption, emission and reflection techniques are all suitable for quantitative and/or qualitative monitoring of chemical changes produced by the sol-gel glasses with entrapped enzymes (col. 4, lines 49-56).

Dunn et al. does not teach explicitly a method of preparing any microanalytical device containing a sol-gel comprising an entrapped biological molecule, or a method of using the microanalytical device.

However, at the filing date of the present application, Avnir et al. already teach obtaining a chemical interaction between at least one reagent trapped in sol-gel glass by doping it with the reagent, and diffusible solutes or components in an adjacent liquid or gas phase. The reagents, the solutes and the components can be any organic or inorganic compounds or materials of biological origin including enzymes (see abstract). Avnir et al. further teach that the doped sol-gel glass can be in any shape suitable for the test, for example, it can have the shape of rods, discs, cubes, sieves, powder or thin films coating conventional glass plates or any other inert solid support (col. 3, lines 20-24). Avnir et al. also teach that the doped sol gel glasses can be used for all chromatographic purposes including liquid, gas and thin layer chromatography. The extraction or separation is performed by passing the solution through columns made from appropriately doped sol gel material (col. 3, lines 445-52[sic]). Particularly, Avnir et al. teach that sol-gel immobilized enzymes, crushed powder sol gel glasses may be used as support for enzymatic column chromatography, with an exemplification showing that the glasses were ground to a size about 60-100 mesh (col. 5, lines 37-39, and col. 7, lines 55-57).

Swedberg et al. already teach a high-throughput microanalysis device having a plurality of sample processing compartments for use in analysis of small and/or macromolecular and/or other solutes in the liquid phase (see abstract). The device is formed by microfabrication of microstructures. Swedberg et al. also teach that the microanalysis device is interfaced with any analytical detection means well known in the art, such as UV/Vis, Near IR, fluorescence, refractive index (RI), Raman techniques, as well as Mass spectrometry (MS) and NMR (col. 6, lines 3-11).

Freeman et al. also teach a miniaturized total analysis system with an in-line NMR detection compartment for the analysis of small and/or macromolecular and/or other solutes in the liquid phase (see abstract).

Accordingly, at the effective filing date of the present application, it would have been obvious for an ordinary skilled artisan in the art to modify the teachings for Dunn et al. by forming a micro-analytical device containing their biological material doped sol-gel and using such micro-analytical device for analysis of small and/or macromolecular and/or other solutes in the liquid phase in light of the teachings of Avnir et al, Swedberg et al., and Freeman et al. because the encapsulated biological material (e.g., enzymes) prepared by the sol-gel process is easier to miniaturize and less cumbersome for use in analytical devices of very high sensitivity such as those taught by Swedberg et al. and Freeman et al. for achieving high throughput sample processing and analysis as well as fast time-to-result analysis of biological liquids in a truly integrated fashion.

An ordinary skilled artisan would have been motivated to carry out the above modification because Dunn et al. already teach that encapsulated biological material (e.g., enzymes) prepared by the sol-gel process is easier to miniaturize and less cumbersome for use in analytical devices of very high sensitivity. Avnir et al. disclose that doped sol gel glasses (including enzyme-doped sol gel glasses are crushed to be used as support for enzymatic column chromatography) for all chromatographic purposes including liquid, gas and thin layer chromatography, and that the micro-devices taught by Swedberg et al. and Freeman et al. that are used for analysis of small and/or macromolecular solutes in the liquid phase allow high throughput sample processing and analysis as well as fast time-to-result analysis of biological liquids in a truly integrated fashion.

An ordinary skilled artisan would have a reasonable expectation of success based on the teachings of Dunn et al., Avnir et al., Swedberg et al. (U.S. Patent No. 5,571,410) and Freeman et al., as well as high level of skill of an ordinary skilled artisan in the art.

Accordingly, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

(Office Action dated February 24, 2004, pages 13-17, emphasis in original)

In the Office Action dated August 11, 2004, claims 45-56, 58, and 59 were rejected under 35 U.S.C. §103(a) as being obvious over Dunn et al. in view of Avnir et al., Swedberg et al., and Freeman et al. for the reasons set forth in the Office Action dated February 24, 2004, and for the following additional reasons:

Applicant's arguments related to the above rejection in the Amendment filed on 5/20/04 (pages 16-18) have been fully considered, but they are respectfully found to be unpersuasive.

Applicant argues mainly that the combined teachings of Dunn and Avnir do not teach or suggest methods of the presently claimed invention for preparing and using a microanalytical device having a sol-gel comprising entrapped biological molecule, and that the teachings of Swedberg and Freeman do not remedy this deficiency. Applicant further argues that that [sic] the mere fact that Dunn touts the benefits of its sol-gel and that Avnir describes the suitability of sol-gels enzymatic column chromatography is insufficient to

suggest to one of skill in the art that the teachings could be modified for use in microanalytical devices with a reasonable expectation of success.

Please note that Dunn clearly teaches that encapsulated or entrapped enzymes are used as micro-catalysts and analytical devices of very high sensitivity, and in contrast to the membrane systems known in the art, encapsulated enzymes in the sol-gel process is far more reliable, less cumbersome and is significantly easier to miniaturize (col. 1, lines 15-36). Avnir further teaches that doped sol-gel glasses can be crushed to be used as support for all chromatographic purposes including liquid, gas, or qualitative and/or quantitative analysis (col. 3, lines 29-61), while both Swedberg and Freeman already disclose micro-devices for a high through-put analysis of small and/or macromolecular solutes in biological fluids. An ordinary skilled artisan would have been motivated to combine the above teachings for achieving a high throughput sample processing and analysis as well as a fast time-to-result analysis of biological liquids in a truly integrated fashion, especially encapsulated enzymes in the sol-gel can be miniaturized [sic] and can be used as support for all chromatographic purposes.

It is not clear why one would not reasonably expect the combined teachings of Dunn, Avnir, Swedberg and Freeman to be successful. Please also note that Applicant has not even actually made or prepared any micro-analytical device comprising a sol-gel comprising an entrapped biological molecule.

Accordingly, the instant claims stand rejected for the reasons of record.
(Office Action dated August 11, 2004, pages 5-6, emphasis in original)

C. Appellant's Arguments Against the Rejections

- (1) *Claims 9, 14, 15, 28-32, and 37-40 are not obvious over Dunn et al. in view of Reetz et al. since prima facie obviousness has not been established*

Appellant has previously argued that the Patent Office has failed to establish a *prima facie* basis for obviousness since neither Dunn et al. nor Reetz et al. teaches or suggests forming a sol in situ in a microanalytical device. Accordingly, appellant has argued that even if these two references were combined, they would not teach or suggest the invention as recited in claim 9. (response filed May 20, 2004, pages 15-16).

Appellant considers this argument to remain valid, notwithstanding the Examiner's rebuttal arguments set forth in the Office Action dated August 11, 2004 (see above). Specifically, in that Office Action, it was asserted that "the term 'the porous inorganic material is formed in situ' includes the the [sic] formation of a porous inorganic material in any reaction vessel." Appellant respectfully disagrees with this conclusion for the following reasons.

As noted above with respect to the language of claim 9 concerning the rejection under the second paragraph of 35 U.S.C. §112, the meaning of the claims includes the reasonable interpretation that the phrase "the porous inorganic matrix is formed in situ" means that the matrix is formed as part of the microanalytical device. This understanding follows from the application disclosure concerning the formation of the matrix at page 6, lines 6-7 and page 7, lines 15-19 of the specification, combined with

the understanding that appellant has disclosed that the term "microfluidic devices" refers to "microanalytical devices" (as noted above, based at least on the disclosure at page 23, lines 7-12). For this reason, the conclusion that the phrase "the porous inorganic material is formed in situ" includes the formation of a porous inorganic matrix in "any reaction vessel" is in error since it clearly conflicts with appellant's disclosure concerning the matrix formation as part of the microanalytical device. It is clearly at odds with the reasonable understanding of the specification and the scope of the claims the skilled artisan would arrive at upon full and complete review of the application disclosure.

Because neither reference teaches or suggests forming a sol in situ in a microanalytical device, it follows that the ordinary artisan could not arrive at the claimed invention solely by reading the disclosures of Dunn et al. and Reetz et al. Accordingly, since the applied references do not teach or suggest the claimed invention, appellant respectfully submits that the obviousness rejection of claims 9, 14, 15, 28-32, and 37-40 over Dunn et al. and Reetz et al. is in error and requests that this rejection be reversed by the Board of Appeals.

Appellant further notes that no motivation exists, and none has been previously suggested by the Patent Office to exist, to modify either Dunn et al., Reetz et al., or the suggested combination of both references, to provide a method as claimed in which the porous inorganic matrix is incorporated into a microanalytical device through in situ formation of the matrix as part of the microanalytical device. Accordingly, the obviousness rejection of claims 9, 14, 15, 28-32, and 37-40 over Dunn et al. and Reetz et al. should also be reversed for this reason.

(2) *Claims 45-56, 58, and 59 are not obvious over Dunn et al. in view of Avnir et al., Swedberg et al., and Freeman et al. since prima facie obviousness has not been established*

Appellant has previously argued that the Patent Office has failed to establish a *prima facie* basis for obviousness since there is no motivation or reasonable expectation of success that the invention of Dunn et al. could be successfully modified for use in microanalytical devices. (response filed May 20, 2004, pages 16-18). These arguments remain valid for at least the following reasons.

Dunn et al. has been cited by the Patent Office for teaching that it is advantageous to encapsulate enzymes in a sol-gel and that such encapsulation is easier to miniaturize and is far less cumbersome than membrane encapsulating systems. The Patent Office has, however, acknowledged that Dunn et al. does not explicitly teach a method of preparing a microanalytical device containing a sol-gel comprising an entrapped biological molecule, or a method of using the microanalytical device (Office Action dated February 24, 2004, page 14).

Appellant submits that there is nothing in Dunn et al. that indicates that any particular advantages would be obtained if the sol-gel was used in a microanalytical device. At best, one skilled in the art might try such a modification of Dunn et al., but there is nothing to suggest that such a modification should be made or that it would or even might be successful.

Avnir et al. is cited by the Patent Office as teaching that doped sol-gel glass can be any shape and that it can be used for any purpose, in particular teaching that crushed powder sol gel glasses may be used as a support for enzymatic column chromatography. The combined teachings of Dunn et al. and Avnir et al. do not suggest the claimed methods of preparing and using a microanalytical device (independent claims 45 and 46) and improved microanalytical device (independent claim 58), however. This failure is not remedied by Swedberg et al. and Freeman et al.

Swedberg et al. is cited by the Patent Office as teaching a high-throughput microanalysis device having a plurality of sample processing compartments for use in the analysis of solutes in the liquid phase. Freeman et al. is further cited as teaching a miniaturized total analysis system with an in-line NMR detection compartment for the analysis of solutes in the liquid phase.

As stated in the Office Action dated August 11, 2004, it would have been obvious to the ordinary skilled artisan to modify Dunn et al. by forming a microanalytical device containing biological material doped sol-gel and using the microanalytical device to analyze solutes in the liquid phase in light of Avnir et al., Swedberg et al., and Freeman et al., because the encapsulated biological material prepared by the sol-gel process is easier to miniaturize and less cumbersome for use in high sensitivity analytical devices as taught by Swedberg et al. and Freeman et al. for achieving high throughput sample processing and analysis.

Appellant respectfully submits that the mere fact that Dunn et al. mentions benefits of its sol-gel and that Avnir et al. describes the suitability of sol-gel enzymatic column chromatography is insufficient to suggest to one of skill the art that Dunn et al. should be modified for use in a microanalytical device, or that any such modification of Dunn et al. could be applied with a reasonable expectation of success in microanalytical devices. Instead, there is no suggestion to make such a modification of Dunn et al. and no reasonable expectation of success in making such a modification to motivate the skilled artisan to incorporate appropriate changes.

As well, both Swedberg et al. and Freeman et al. are directed to microanalytical devices having a unique configuration. Sample treatment, as well as analytical separation and detection, are described generically, as they appear to be of less significance to the invention than the device's configuration *per se*. Therefore, Swedberg et al. and Freeman et al. also do not provide the necessary motivation to modify

Dunn et al. for use in a microanalytical device.

Appellant further considers the foregoing arguments to remain valid, notwithstanding the Examiner's rebuttal comments set forth in the Office Action dated August 11, 2004 (see above). Specifically, in that Office Action, it was re-asserted that the "skilled artisan would have been motivated to combine the above teachings for achieving a high throughput sample processing and analysis as well as a fast time-to-result analysis of biological liquids in a truly integrated fashion, especially encapsulated enzymes in the sol-gel can be miniaturized [sic] and can be used as support for all chromatographic purposes." Appellant respectfully disagrees with this conclusion for reasons noted above, and for the following reasons.

Briefly stated, Dunn et al. concerns the formation of a sol-gel encapsulated enzyme, but does not disclose or suggest a method according to claim 44 of "placing the sol-gel in or on [a] microanalytical device." Dunn et al. also does not disclose or suggest "forming the sol-gel into a bed within [a] microanalytical device or on the surface of the microanalytical device" according to claim 46. Further, Dunn et al. does not disclose or suggest the improvements in a microanalytical device according to claim 58 in which a sol-gel having a biological molecule entrapped therein is incorporated into at least one feature selected from microchannels, microcolumns, and combinations thereof, and/or onto a surface of a substrate of a microanalytical device comprised of the substrate and the at least one feature, wherein the sol-gel is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.

These missing elements of Dunn et al. have been acknowledged and agreed with by the Patent Office (Office Action dated February 24, 2004, page 14). Furthermore, there is no disclosure or suggestion in any of the applied secondary references of a motivation to modify Dunn et al. according to appellant's claims. At best, Avnir et al., Swedberg et al., and Freeman et al. merely provide disclosures concerning the possible uses of certain sol-gels, but do not suggest any reason to modify or apply the sol-gel of Dunn et al. according to appellant's claims. Instead, the information provided by these secondary references amounts only to an "obvious to try" assertion that is not supported by a showing of appropriate motivation and reasonable expectation of success to satisfy the burden of establishing a *prima facie* obviousness argument. Stated another way, while Avnir et al., Swedberg et al., and Freeman et al. do provide some information concerning the possible applications for sol-gels, the Patent Office has not articulated an appropriate reason concerning why these references provide a suitable motivation to include the features of appellant's claims in the invention of Dunn et al. to justify a rejection of the claims under 35 U.S.C. §103(a).

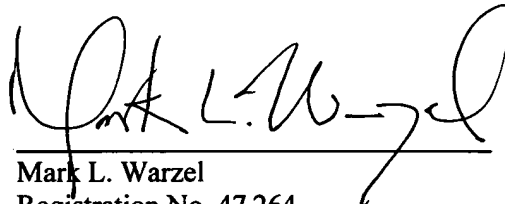
Because the secondary references, Avnir et al., Swedberg et al., and Freeman et al., do not provide a reasonable motivation to modify Dunn et al., nor a reasonable expectation of success in carrying out such a proposed modification, a proper *prima facie* basis for rejection of the claims has not been set forth. Accordingly, appellant respectfully submits that the obviousness rejection of claims 45-56, 58, and 59 over Dunn et al. in view of Avnir et al., Swedberg et al., and Freeman et al. does not satisfy the requirements of 35 U.S.C. §103(a). Reversal of this rejection by the Board of Appeals is requested.

Conclusion

For the foregoing reasons, appellant respectfully requests that the Board of Appeals reverse the Patent Office's rejection of claims 9, 14, 15, 28-32, and 37-40 under 35 U.S.C. § 112, second paragraph, and the rejection of claims 9, 14, 15, 28-32, 37-40, 45-56, 58, and 59 under 35 U.S.C. §103(a).

Respectfully submitted,

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APPENDIX A

Listing of the Claims

1. A method for immobilizing a biological molecule in a porous inorganic matrix, said method comprising:
 - forming an aqueous composition comprising a ceramic oxide colloidal sol mixed with an acidified oxide salt solution;
 - adding to said composition an amount of the biological molecule in a physiologically acceptable-buffered solution, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;
 - shaping the gel produced in step (b) into a final form;
 - aging the gel; and
 - crushing the aged gel into particulates, wherein the crushed gel particulates are between about 10 μm and about 80 μm in diameter and are suitable for incorporation into a microanalytical device;
 - wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained.
2. The method of claim 1, wherein the gel is aged for about two weeks.
3. The method of claim 1, wherein the gel is aged at a temperature of from about 4°C to about 40°C.
4. The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 6.0 and about 8.5.
5. The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 4.0 and about 7.0.
6. The method of claim 1, wherein the pH of the mixture after the addition of the biological molecule is between about 7.0 and 9.0.
9. A method for immobilizing a biological molecule in a porous inorganic matrix incorporated into a microanalytical device, said method comprising:
 - forming an aqueous composition comprising a tetraalkyl orthosilicate and a silane, wherein the

silane is substituted with a C₈-C₂₄ alkyl group and substituted with at least two leaving groups selected from OR and halo, mixed with an acidified oxide salt solution;

adding to said composition an amount of the biological material in a physiologically acceptable-buffered solution wherein the resulting aqueous composition has a pH ranging from about 6 to about 8.5, said aqueous composition becoming turbid on being transformed into a polymerizing hydroxide solution and transforming to a gel;

shaping the gel produced in step (b) into a final form; and

aging the gel;

wherein said biological molecule is entrapped within pores of the gel, and the activity of the biological molecule is retained; and wherein the porous inorganic matrix is formed in situ.

14. The method of claim 9, wherein the alkyl group is C₁₈.

15. The method of claim 9, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.

16. The method of claim 1, wherein the sol is comprised of colloidal silica sol and a dissolved metal silicate.

17. The method of claim 16, wherein the metal silicate is sodium silicate.

18. The method of claim 1, wherein the sol comprises a tetraalkyl orthosilicate and a silane substituted with at least two leaving groups selected from the group consisting of OR and halo.

19. The method of claim 18, wherein the silane is substituted with a C₈-C₂₄ alkyl group.

20. The method of claim 19, wherein the alkyl group is C₁₈.

21. The method of claim 18, wherein the tetraalkyl orthosilicate is selected from the group consisting of tetra-ethyl orthosilicate, tetra-methyl orthosilicate, and combinations thereof.

24. The method of claim 1, wherein the particle size of the ceramic oxide colloidal sol is selected to produce pores when the gel is aged, said pores being of a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.

26. The method of claim 24, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.
27. The method of claim 1, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.
28. The method of claim 9, wherein the pores have a diameter which is approximately the same as the diameter of the biological molecule to be entrapped.
29. The method of claim 28, wherein the diameter of the pores is less than the diameter of the entrapped biomolecule.
30. The method of claim 9, wherein the gel produced in step (b) is shaped into forms selected from the group consisting of a monolithic gel, thin film, or fiber.
31. The method of claim 9, wherein the pores have an average diameter ranging from about 1 nm to about 100 nm.
32. The method of claim 31, wherein the pores have an average diameter ranging from about 2 nm to about 50 nm.
33. The method of claim 24, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.
34. The method of claim 24, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.
35. The method of claim 24, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.
36. The method of claim 24, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.

37. The method of claim 28, wherein molecules having a mass of 3,000 Da or less can diffuse through the pores.
38. The method of claim 28, wherein molecules having a mass of 5,000 Da or less can diffuse through the pores.
39. The method of claim 28, wherein molecules having a mass of 10,000 Da or less can diffuse through the pores.
40. The method of claim 28, wherein molecules having a mass of 15,000 Da or less can diffuse through the pores.
41. The method of claim 1, wherein the biological molecule is selected from the group consisting of polynucleotides, enzymes, antibodies, coagulation modulators, cytokines, endorphins, peptidyl hormones, kinins, receptors, genes, gene fragments, cell fragments, membrane fragments, and solubilized membrane proteins.
42. The method of claim 41, wherein the enzyme is selected from the group consisting of RNase, DNase, telomerase, ligase, nuclease, ribonuclease; hydrogenase, dehydrogenase, aldase, amidase, aminotransferase, amylase, anhydrase, apyrase, arginase, aspartase, aspariginase, carboxylase, carboxypeptidase, catalase, cellulase, cholinesterase, acetylcholinesterase, deaminase, dextranase, dismutase, elastase, esterase, fumarase, glucosidase, hexokinase, isomerase, invertase, kinase, lactase, lipase, lysozyme, malase, naringinase, oxidase, oxygenase, papain, pectinase, peptidase, pepsin, peroxidase, phosphodiesterase, phosphotase, protease, reductase, transferase, tyrosinase, urase, trypsin, chymotrypsin, hydrolases, isomerases, proteases, ligases and oxidoreductases such as esterases, phosphatases, glycosidases and peptidases, superoxide dismutase, tissue plasminogen activator, renin, adenosine deaminase, alpha-glucocerebrosidase, asparaginase, dornase-alpha, hyaluronidase, elastase, trypsin, thymidine kinase, tryptophan hydroxylase, urokinase, kallikrein, bromelain, cathepsins B, D, G, C, clostripain, endoproteinase Arg C, endoproteinase Asp N, endoproteinase Glu C, endoproteinase Lys C, Factor Xa, proteinase K, subtilisin, thermolysin, acyloamino acid releasing enzyme, aminopeptidases, carboxypeptidases, and pyroglutamate aminopeptidase.
43. The method of claim 1, wherein the colloidal sol particle size is from about 1 nm to about 30 nm.

44. A method of preparing a microanalytical device, comprising forming a sol-gel comprising an entrapped biological molecule, crushing the sol-gel to particulates having a diameter of from about 10 μm to about 80 μm , and forming the sol-gel particulates into a bed within the microanalytical device or on the surface of the microanalytical device.
45. A method of preparing a microanalytical device comprising forming a sol-gel comprising an entrapped biological molecule, wherein the form of said sol-gel is selected from the group consisting of a monolithic gel, thin film, or fiber and wherein the sol-gel is placed in or on the microanalytical device.
46. A method of using a microanalytical device comprising a sol-gel comprising an entrapped biological molecule, comprising forming the sol-gel into a bed within the microanalytical device or on the surface of the microanalytical device, applying an analyte sample to the bed, optionally applying additional buffer solution to the bed, and analyzing the eluant from the bed.
47. The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microcolumn or microchannel.
48. The method of claim 44 or 46, wherein the bed on the microanalytical device is in the form of a microarray.
49. The method of claim 46, wherein the eluant is analyzed using mass spectrometry.
50. The method of claim 46, wherein the eluant is analyzed using micro or capillary electrophoresis.
51. The method of claim 46, wherein the interaction of any component in the sample with the entrapped biological molecule in the sol-gel is measured using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index and Raman spectroscopies.
52. The method of claim 46, further comprising washing the sol-gel with a solution to elute analytes from the sol-gel, and analyzing the analytes.
53. The method of claim 52, wherein the analytes are analyzed using mass spectrometry.

54. The method of claim 52, wherein the analytes are analyzed using a method selected from the group consisting of UV/Visible, Near IR, fluorescence, refractive index and Raman spectroscopies.
55. The method of claims 44 or 45, wherein the microanalytical device is fabricated by a method selected from the group consisting of silicon micromachining, microlithography, molding and etching.
56. The method of claim 45, wherein the sol-gel is formed in situ on the microanalytical device.
58. In a microanalytical device comprised of a substrate and at least one feature selected from microchannels, microcolumns, and combinations thereof, the improvement which comprises incorporating into said at least one feature and/or onto a surface of the substrate a sol-gel having a biological molecule entrapped therein, wherein the sol-gel is in a form selected from the group consisting of a monolithic gel, a thin film, and a fiber.
59. The microanalytical device of claim 58, adapted for performing high throughput screening of samples.